Summary of Progress on Field Guide Revision

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Field Guide for the Determination of Biological Contaminants in Environmental Samples

AIHA Biosafety Committee

American Industrial Hygiene Association 1996

Approaches

- Involve more professional authors and reviewers
- Foster a consensus in state-of-the-art sampling and analysis methodology to present to practicing industrial hygienists
- Seek a balanced approach in providing background information that will guide the users in answering questions related to the application of methods

Do a Better Job of

- Explaining factors to consider when taking indoor and outdoor air samples
- Explaining how statistics may be applied to the data collected
- Outlining what steps are reasonable based on the data obtained
- Explaining strengths and weaknesses of different kinds of air and bulk samples
- Providing information on common building-associated microbes

Chapters in 1996 Field Guide [1]

- Introduction
- Disease Caused by Bioaerosols
- Viable Fungi and Bacteria in Air, Bulk, and Surface Samples
- Viable Air Sampling Instruments: Description and Operation Procedures
- *Legionella* Bacteria in Air and Water Samples

Chapters in 1996 Field Guide 2

- Total (Viable and Nonviable) Fungi and Substances Derived from Fungi in Air, Bulk, and Surface Samples
- Substances Derived from Bacteria in Air, Water, and Bulk Samples
- Allergens

Chapters in 2004 Revision

- Introduction
- Elements of Building Science **
- Microbial Ecology **
- Health Effects
- Planning and Conducting a Survey**
- Culturable Fungi and Bacteria
- Total Fungi and Other Substances
- Allergens
- Air Sampling Instruments

Elements of Building Science

- Chapter focuses on how, where, and why buildings “fail”
- Moisture sources in buildings
- Heat, air and moisture flows and storage
- Measuring moisture

Elements of Building Science 2

Moisture sources in buildings

- Wetting incidents
- Chronic moisture problems

Elements of Building Science 3

- Heat, air and moisture flows and storage
  - Diffusion of water vapor
  - Wicking of liquid water through materials
  - Flows of liquid water through cracks and openings
  - Inflows of humid air in hot weather

Elements of Building Science 4

- Heat, air and moisture flows and storage
  - Outflows of humid air in cold weather
  - Wind driven rain and exterior cladding leaks
  - Wet surfaces in or near humidification or dehumidification systems

Microbial Ecology

- **Types of bacteria and fungi that would be expected in certain moisture conditions (i.e., *Stachybotrys* on chronically wet cellulose materials)**
- **Succession of species over time and changing conditions**

Health Effects

- **Drafted by MD, CIH**
- **Positions environmental measurements in terms of anticipated outcomes and defines risk groups**

Health Effects 2

- **Essentially an annotated comment on the health messaging of building-associated fungi and bacteria**
  - **derived from ACGIH, EPA, and Institute of Medicine documents**
  - **very much in sync with the AIHA "The Facts About Mold":**

Allergy is the most prevalent health condition

Planning and Conducting A Survey

- Informed Inspection
- Sampling Strategy
- Statistical Analysis
- Laboratory Selection

Informed Inspection

- **“Informed”**
  - **Knowledge of how building “operates”**
  - **Knowledge of “population” and health status**
- **Thorough inspection of building components**
  - **Water damage and visible mold growth**
  - **Amount of visible mold growth on building materials - *the best environmental estimate of health risk***
  - **When and where to sample**

Sampling Strategy 1

- **Why Sample?**
  - **Complaint investigation**
  - **Source identification**
  - **Exposure documentation**
  - **Selection and evaluation of remediation or controls**

Sampling Strategy 2

- When and Where to Sample?
  - Spatial variables
  - Temporal variables
  - Indoor vs. outdoor comparisons
  - Complaint vs. non-complaint areas

Sampling Strategy 3

- **What Kind of Samples Do I Take?**
  - **Air sample**
  - **Surface sample**
  - **Settled dust**
  - **Bulk sample**

Air Sampling for Bacteria

- **Cautious recommendation against taking such samples**
- **Limited basis for interpretation**
- **For *Legionella,* reference current ASHRAE and CDC views**
- **Endotoxin sampling indoors may become more common during the shelf life of the 2004 guide**

Air Sampling for Fungi

- **Provides no information on exposure whether obtained by either “total” or “viable” methods**
- **AIHA “The Facts about Mold”: more useful in larger buildings - greater potential to miss hidden contamination**
- **1996 version of Field Guide placed emphasis on *indicator species* and *relative prevalence of species* data from concurrent outdoor air samples taken above grade**

Surface Samples

- **Swab**
- **Tape-Lift or Cellotape**
- **Useful for documenting whether or not mold colonization occurs on a surface**
- **Presence of some spores and a few hyphal fragments not evidence of fungal colonization**

Settled (or Transported) Dust [1]

- Microbial agents once airborne
  - Caution--same rank order may not occur
  - Represents history of exposure?
- Microbial contamination of porous surfaces
- Dust mite, cat, and cockroach allergens useful in assessing allergic risk (IOM, 1993; ACGIH, 1999)
  - Why not sample for fungal allergens?

Settled (or Transported) Dust 2

- **Fungal biomass in dust estimated by β-glucan or ergosterol content**
  - **compared to presence of other microbial agents or to health effects data (Douwes, *et al.,* 2000; Saraf, *et al.,* 1999).**
- **Dusts with preponderant cellulose degrading fungi or *Penicillium* and *Aspergillus* species**
  - **Indication of water or dampness (forensic use) (Dillon, *et al*., 1999; Morey, 1995, 2001).**

Bulk Samples

- Assess extent of microbial contamination
- Determine species of fungi growing

Statistics

- Hypothesis Testing and Significance Level
- Errors in Bioaerosol Measurements
  - Random error
  - Bias
- Determining number of samples to collect
- Calculating sample size
- Sampling to determine acceptability

Strategies to Improve Accuracy and Representativeness

- Duplicate measurements (minimum)
- Averaging times as long as possible
- Multiple sampling locations (spatial variability)
- Multiple times of day (morning and afternoon) and times of year (heating and cooling periods) (temporal variability)
- Ensure accuracy and precision of method
  - Written procedures, positive and negative controls

Total Fungi and Other Substances[1]

- Total Fungal Spore and Hyphal Fragment Counts (Air-O-Cell Cassettes)
- Quantitative Polymerase Chain Reaction (QPCR)
- Microbial Volatile Organic Compounds (MVOCs)

Total Fungi and Other Substances[2]

- Mycotoxins
- Endotoxins
- Glucans
- Ergosterol
- Fungal Extracellular Polysaccharides (EPS)

Quantitative Polymerase Chain Reaction (QPCR)

- Haugland, R.A., Vesper, S.J., and L.J. Wymer
  - Quantitative determination of *S. chartarum* spores
  - Real time, fluorescent probe-based detection of PCR products
  - Detection limit = 2 spores
  - Reasonable agreement with microscopy
- Methodology expanded to determination of about 100 other molds

Microbial Volatile Organic Compounds (MVOCs)

- **Low molecular weight chemicals that are fungal and bacterial metabolites**
- **Ability to diffuse from enclosed spaces or off HVAC filters free of visible contamination make them indicators of latent mold growth (Strom *et al.*, 1994; Elke *et al.*, 1999; Ahern *et al.*, 1997)**

MVOCs

- **Pure culture studies reveal anywhere from c.25-196 MVOCs of a variety of chemical types:**
  - **alcohols, aldehydes, ketones, esters, ethers, aromatics, amines, terpenes, furans, and sulfur-containing compounds**
- **Suspected of sensory irritation but no compelling proof (Kreja and Seidel, 2002a)**

Fungal Extracellular Polysaccharides (EPS)

- **Secreted through the cell walls of fungi**
- **Contain repeating units of either the same or different (e.g., galactose, mannose, or glucose saccharides)**
- **Immunogenicity fairly specific by genus with exception: cross-reactivity between the *Aspergillus* and *Penicillium***

EPS as a Surrogate Marker of Fungal Exposure

- **Fungal EPS ELISA methodology used in assessment of fungal exposure in residential environments (Chew, et al. 2001; Douwes, et al. 1999; Wouters, et al., 2000)**
- **House dust samples vacuumed/analyzed for the cross-reactive Asp/ Pen EPS**
- **Positive associations between EPS and occupant reports of dampness and mold**

Allergens in Bulk Dust

- Fungal, Dust Mite, Cockroach, Cat/Dog, Pollens
- When is Testing Indicated?
  - Symptoms linked to allergen exposure
  - Predisposing situations
    - water damage
    - elevated temperature (> 28C or 82F) and

      RH(> 60-65%)
    - carpeting
    - pets or animals housed in the building

Allergen Sample Collection Devices

- **Bulk Samples**
  - Vacuum Cleaner with Bag
  - Vacuum Cleaner with Attachments
- **Air Samples**
  - Filter Cassette with Polycarbonate Membrane filter
  - Personal and Area

Air Sampling Instruments 1

- Glass impingers
  - SKC Biosampler
  - All-Glass Impinger (AGI-30)
- Multi-Holed Impactors
  - SAS Sampler
  - N6 One-stage Impactor
  - Two-stage Impactor

Air Sampling Instruments 2

- Slit Impactors
  - Slit-to-Agar Impactor
  - Spore Traps
    - Air-O-Cell
    - Allergenco-moving slide
    - Burkhardt
    - INHALIX Nasal Sampler
- Centrifugal Samplers
  - Reuter Centrifugal Sampler (RCS)

Summary of Revisions

- Provide more information on the physical inspection process
- Re-emphasize that air samples should not be taken unless a physical inspection has been done or initiated
- Place the information together in a more user-friendly fashion
- Provide more information on emerging field methods

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